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Research Article

IN VIVO STUDY OF ANTIDIARRHOEAL ACTIVITY OF DAIRY WASTE WHEY

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ABSTRACT

Whey powder produced out of dairy waste whey was analyzed for its antidiarrhoeal activity in Wistar albino rats. The study was conducted at various doses which showed a remarkable antidiarrhoeal activity evidenced by the reduction in the rate of defecation and consistency of faeces. Results are comparable to that of standard drug loperamide. Like loperamide (3 mg kg^{-1} body weight), a single dose of whey powder (250 mg, 500 mg kg^{-1} body weight), produced significant decrease in the severity of diarrhoea induced by castor oil. The experimental findings showed that dairy waste whey possess significant antidiarrhoeal activity and may be a potent source of antidiarrhoeal drug in future.

Key words: Albino rats, Antidiarrhoeal activity, Dairy waste whey, castor oil, loperamide.

INTRODUCTION

In developing countries, a majority of people living in rural areas almost exclusively use traditional medicines in treating all sorts of diseases including diarrhoea. Diarrhoea is a major health problem, especially for children under the age of 5 and up to 17% of children admitted in the pediatric ward die of diarrhoea. Worldwide distribution of diarrhoea accounts for more than 5-8milloin deaths each year in infants and children below 5 years old especially in developing countries[1]. According to the WHO's Department of Food Safety, the new estimate 2001, "Secretary diarrhoea is the most dangerous symptoms of gastrointestinal problems and is associated with diarrhoea kills around 2.2 million people each year and infects around 4 billion people in a year". Most deaths occur in children particularly due to dehydration. Excessive defecation and stool outputs abnormally loose consistency was observed[2]. The incidence of diarrhoeal disease still remains high despite the efforts of many governments and international organizations to curb it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs which are not always free from adverse effects[3]

India is a number one producer of milk in the world and it produces 150 million tons per year. Thousands of dairies are handling this milk. Raw milk is collected from villages and transported to metro dairies for the purpose of processing and conversion of products such as butter, ghee, paneer, cheese, peda, yoghurt, curd, buttermilk, ghoa, etc. Especially during the preparation of cheese and paneer, huge quantities of whey are wasted in gutter as a dairy waste. This whey has nutritional and medicinal properties. Whey is the liquid resulting from the coagulation of milk and is generated from cheese manufacture. About nine liters of whey is generated for every kilogram of cheese manufactured, and a large cheese making plant can generate over 1 million liters of whey daily[4]. Previously whey was not used for humans and was fed to pigs or other livestock, spread as fertilizer or simply thrown out[5]. Whey is a potent pollutant with a biological oxygen demand of 35-45 g L^{-1}. 4,000 L whey output of a small creamery has the polluting strength equal to sewage of 1,900 people[6]. Nowadays whey is evolving into a product because it contains lactose, minerals and proteins as well as its functional properties to food[7]. The composition of whey and whey products depends on the methods of production, purification, and concentration used. Table 1 shows an average protein composition of sweet whey proteins.

Until recently, sweet whey has been discarded. Recovering the solid components of whey is attractive for two main reasons, to reduce the organic pollution created by whey wastes when they are discarded[10] and mostly for optimal utilization of the nutritional and functional properties offered by whey proteins. The microbicidal activities of lactoferrin and some of the peptides derived from lactoferrin are of interest, since they were found to kill some pathogenic microorganisms such as gram-positive bacteria[11, 12]. In general, protein profiles for heat-treated skim milk indicate that the denaturation of the total proteins begins at 40 °C, accelerates with increasing temperature, and becomes 95% complete at 85 °C[9]. The whey proteins, individually and in solution, denature between 64 and 85 °C: at neutral pH, α-LA denatures at 64 °C and β-LG denatures at 78 °C; above 85 °C, the whey proteins start to aggregate and gel[13]. Changes in protein structure that occur during denaturation were shown to be irreversible [14, 15].

Table 1: Sweet whey proteins: Typical composition and physical properties[8, 9].

Protein	Content %	Molecular Weight (Kg/mol)	Isoelectric pH
β-Lactoglobulin (β-LG)	48-58	18	5.4
α-Lactalbumin (α-LA)	13-19	14	4.4
Glycomacropeptide (GMP)	12-20	8.6	<3.8
Bovineserum albumin (BSA)	6	66	5.1
Immunoglobulin (Igs)	8-12	150	5-8
Lactoferrin (LF)	2	77	7.9
Lactoperoxidas	0.5	78	9.6

Hence in the present study whey was collected and subjected to for powder preparation in the laboratory condition and the powder is subjected for its antidiarrhoeal activities against experimentally induced diarrhoea in albino rats.

MATERIALS AND METHODS

Treatment of amimals

Adult albino rats of both sex (Wistar strain) weighing 150-250 g were used in the pharmacological and toxicological studies. The inbred animals were taken from animal house in C. L. Baid Metha College of Pharmacy, Chennai, India. The animals were maintained in well ventilated room temperature with natural 12 ± 1 h day and night cycle in the wire-bottomed cages. They were fed balanced rodent pellet diet from Poultry Research Station, Nandanam, Chennai, India and tap water (boiled, cooled) was provided throughout the experimental period. The animals were sheltered for one week and prior to the experiment they were acclimatized to

laboratory temperature. The protocol was approved by Animal Ethics Committee constituted for the purpose as per Committee for the Purpose of Control and Supervision on Experiments on Animals Guidelines. All studies were carried out 4 groups of 6 Rats each. Each rat was housed separately in a metabolic cage.

Preparation of whey powder

During the preparation of whey liquid the entire protein content and the other peptides were retained. One liter of milk is maintained at 4 °C overnight and to it 15 mL of 20% food grade lactic acid was added and stirred well with clean knife. To that 0.001% of rennet powder was added and mixed well. This mixture of milk was kept in luke warm water undisturbed for nearly 30-40 min.

After the incubation time the curd was formed at the top and the whey liquid at the bottom. The curd was cut with knife and the whey liquid was oozed out from the bottom. The whey liquid was filtered with the cheese cloth to remove any tiny particles of casein present in the whey. The whey collected was a clear liquid and cooled to 4 °C. As soon as it was collected it was powdered using the freeze drier. About 800 mL of the whey liquid was collected from 1000 mL of the milk, by using this whey liquid 50 g of whey powder was prepared.

Acute Toxicity

Acute toxicity studies were conducted for the prepared whey powder in Wistar albino rats by Staircase method. Step wise dose of whey powder dissolved in water from 50 mg kg^{-1} body weight up to the dose 5000 mg kg^{-1} body weight caused no considerable signs of toxicity in the tested animals. One tenth of the upper dose was selected as the levels for examination of antidiarrhoeal activity.

Drugs and Chemicals

Loperamide (Standard reference antidiarrhoeal drug), castor oil (Laxative agent) and normal saline solution (0.9% NaCl).

Experimental protocol for castor oil-induced diarrhoea

24 Albino rats were allowed to fast for 18 hours and divided into 4 groups of 6 animals each. Group I received castor oil at dose of 1 mL kg^{-1} p.o. for the induction of diarrhoea[16]. Group II received the standard drug Loperamide 3 mg kg^{-1} p.o after 30 min of receiving castor oil. Group III received whey sample of 250 mg kg^{-1} p.o after 30 min of receiving castor oil. Group IV received whey sample of 500 mg kg^{-1} p.o after 30 min of receiving castor oil.

Animals of all groups were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the severity of diarrhoea was assessed hourly for 4 hours. The total number of faeces excreted and total weight of the faeces were recorded within the period of 4 hours and compared with the castor oil fed group (Fig 1.). The mean of the stools passed by the treated groups were compared with positive control animals that were given castor oil (1 ml kg^{-1} B.W) p.

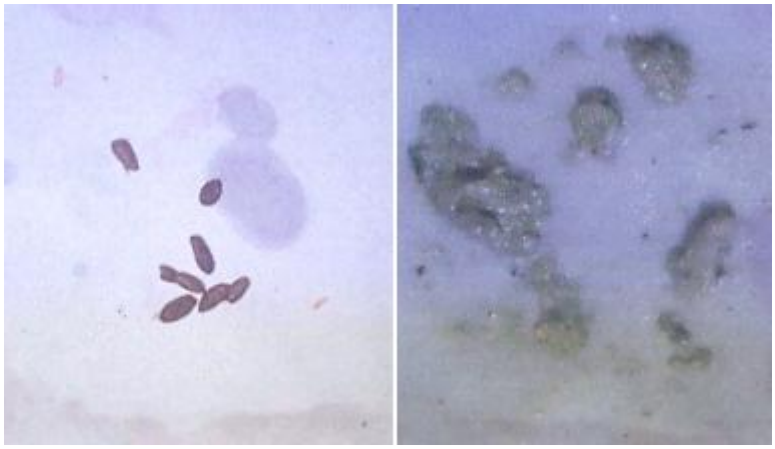

Fig 1.Comparison of normal (left) and diarrhoeal faeces (right)Statistical analysis

The values were expressed as mean ± Standard Error Mean (n = 6). The statistical analysis of data was done by Analysis of Variance (ANOVA) using SPSS package. A One-way ANOVA enabled the significant differences between the values to be observed. The post hoc Dunnett's test was used to identify these differences.

RESULTS

Effect of whey powder on castor oil induced diarrhoea

In the castor oil induced diarrhoea experiment, the rats that did not receive the Whey sample, showed typical diarrhoea signs such as watery and frequent defecation. Whey protein produced a marked antidiarrhoeal effect in the rats. From Table 2 and 3 both doses of Whey significantly decreased the total number of wet faeces produced by administration of castor oil. It was observed that decrease in the total number of faeces of 7.5 at the dose of 500 mg kg^{-1} and 5.25 at the dose of 250 mg kg^{-1} as compared to the castor oil-treated control group which showed a total number of 11.5 faeces. The percentage of inhibition of castor oil induced diarrhoea in whey protein treated rats was 34.78 and 54.34% respectively at 500 and 250 mg kg^{-1} dose respectively.

Table 2: Effect of whey on castor oil-induced diarrhoea for inhibition of total no. of faeces

	Group I Castor oil induced	Group II Loperamide (3 mg kg^{-1})	Group III 250 mg kg^{-1} whey + castor oil	Group IV 500 mg kg^{-1} whey + castor oil
Total no. of faeces	17.25 ± 1.49	7.75 ± 0.95	8 ± 0.91 ^{a***}	10.50 ± 1.32 ^{b***}
Total no. of diarrhoeal faeces	11.5 ± 1.04	4 ± 0.41	5.25±0.46 ^{a***}	7.5 ± 0.65 ^{b***}
Inhibition (%)	0	65.21	54.34	34.78

All the values are expressed as mean ± SEM (n=6).
Statistical Significance was calculated by ANOVA followed by post hoc Dunnett's using SPSS package.
NS – Not Significant *** p > 0.001 ** p > 0.01 * p > 0.05
a- Group I vs. Group III b- Group I vs. Group IV

Table 3: Effect of whey on castor oil-induced diarrhea in terms of total weight of faeces

	Group I Castor oil induced	Group II Loperamide (3 mg kg^{-1})	Group III 250 mg kg^{-1} whey + castor oil	Group IV 500 mg kg^{-1} whey + Castor oil
Total weight of faeces	8.99 ± 0.18	3.55 ± 0.13	3.59 ±0.16	4.40 ± 0.29
Inhibition (%)	0	60.51	60.07	51.06

All the values are expressed as mean ± SEM (n=6).

The antidiarrhoeal effect of whey protein was similar to that of the standard drug, Loperamide (3 mg kg^{-1}) which produced an inhibition of 65.21%. The average weight of faeces in the castor oil induced group was 8.99 g. Treatment with 500 mg kg^{-1} and 250 mg kg^{-1} of whey protein reduced the weight of faeces to 4.4 g and 3.59 g respectively. The percentage of inhibition in terms of total weight of the faeces is 60.53 in loperamide and 60.05 in 250 mg mL^{-1} of whey.

Discussion

In developing countries the quarter of infants and childhood mortality is related to diarrhoea. The highest mortality rates have been reported to be in children less than 5 years of age. During the past decade oral dehydration therapy has reduced mortality from acute diarrhoeal disease, whereas chronic diarrhoea remains a life threatening problem in those regions, in which mal-nutrition is a common co-existing and complication factors. Castor oil causes diarrhoea due to its active metabolite ricinolic acid[17, 18] which stimulates peristaltic activity in the small intestine, leading to changes in the electrolytic permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin[19].

The results of the present study showed that the whey powder produced a statistically significant reaction in the severity and frequency of diarrhoea produced by castor oil (Fig 2., Fig 3.). Its effect depends on the low dose of (250 mg kg^{-1}) has greater impact than that of higher dose of (500 mg kg^{-1}). The results were similar to that of the standard drug loperamide (3 mg kg^{-1}) with regard to the severity of diarrhoea. Whey contains several unique components with broad antibacterial properties including Immunoglobulins (Igs), Lactoferrin (Lf), lactoperoxidase (Lp), Glycomacropeptide (GMP) and Spingholipids. Significant levels of these compounds have been shown to survive passage through the stomach and small intestine and arrive as intact proteins in the large intestine, where they exert their biological effect[20]. Perhaps the best known of the whey components that provide antimicrobial action in the intestinal tract are the Igs.

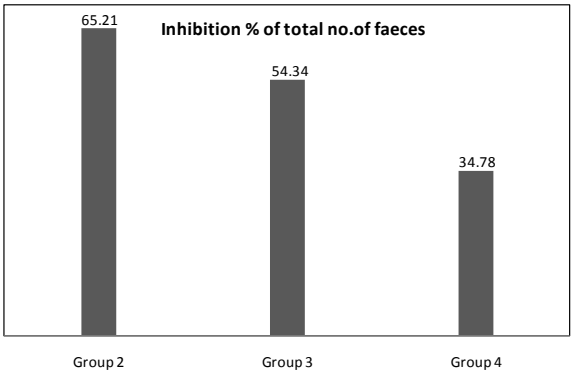

Fig. 1: Effect of whey on castor oil-induced diarrhoea for inhibition of total no. of faeces.

Igs falls into several classes including IgG. IgM and IgA. IgG predominate in milk derived sources such as whey and can traditionally comprise up to 1% of the total weight of the whey proteins. IgG has been shown to bind the toxin produced by clostridium difficile, thereby reducing much of the deleterious effects of infection, including diarrhoea, dehydration, muscle aches[20]. GMP has also been shown to inhibit cholera toxin binding to receptors in the intestinal tract[21, 22]. Lactoferrin an iron binding protein is derived compound that has gained recognition for its antibacterial properties. Studies have shown that most lactoferrin survives passage through stomach and small intestine and arrives in the lower bowel. Where it can sequester iron from bacteria[23]. Since pathogens in particular have high iron requirements for metabolism and growth, this property of lactoferrin makes it broadly antimicrobial in nature. Lactobacilli can utilize lactoferrin bound iron, allowing lactoferrin to inhibit pathogenic bacteria.

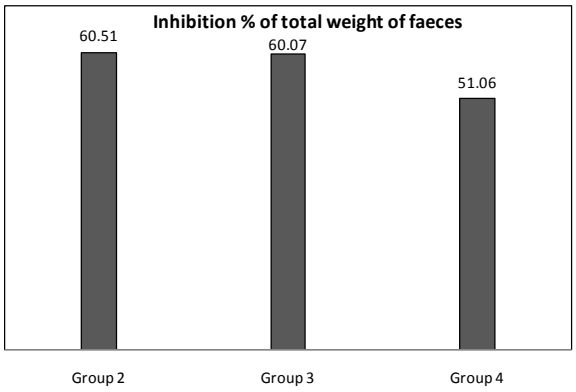

Fig. 2: Effect of castor oil induced diarrhoea for total weight of faeces.

CONCLUSION

The present study has provided new insights into the antidiarrhoeal action of the waste whey powder against castor oil induced diarrhoea. The results of this investigation showed that whey powder prepared waste whey retains pharmacologically active substance with antidiarrhoeal properties. Future research will be carried out to find out the active ingredients that are responsible foe antidiarrhoeal activity.

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